

WHAT IS CLAIMED IS:

1. An oligodeoxynucleotide (ODN) library comprising a plurality of oligodeoxynucleotides of specific length, at least one of the oligodeoxynucleotides comprising said ODN library being capable of interacting with a target genomic DNA, mRNA or protein when inserted into a DNA expression vector with the specific calling sequence for said oligodeoxynucleotide being embedded in said expression vector, said expression vector being capable of being introduced into a target cell to produce at least one of said oligodeoxynucleotides when induced by exposure to a chemical agent for interacting with genomic DNA, mRNA or protein with observable result.

2. A process for identifying and isolating an oligodeoxynucleotide comprising the steps of:

utilizing the ODN library of claim 1 to express a plurality of copies of at least one said oligodeoxynucleotide in the target cell;

growing the target cells into a colony of cells;

dividing the colony into paired colonies;

exposing one of the paired colonies to a chemical agent capable of inducing expression of said at least one oligodeoxynucleotide by the cells of the exposed colony, causing the expressed oligodeoxynucleotide to interact with genomic DNA, mRNA or a protein to alter expression of a gene;

observing the result in said exposed cells; and

sequencing the DNA of the cells of the unexposed colony to identify the sequence of the library oligodeoxynucleotide that caused alteration of the gene.

3. The method of claim 2 wherein said cells are bacteria strain DH5 α Pro.

4. The plasmid pssXG.

5. The plasmid of claim 4 comprising a PBS having the sequence 5'-TGGTGCCTCCGAG-3' [Seq. ID No. 3].

6. A cell having the plasmid of claim 4 transformed therein.

7. A prokaryotic cell having the plasmid of claim 4 transformed therein.

8. The plasmid of claim 4 comprising a sequence coding for *in vivo* expression of a single-stranded DNA enzyme targeted to the mRNA transcript of the bacterial FtsZ gene.

9. The plasmid of claim 8 wherein the single-stranded DNA enzyme is specific for a GU site at position 880 of the bacterial FtsZ gene.

10. The plasmid of claim 8 wherein the single-stranded DNA enzyme comprises 5'-N₁-GGCTAGCTACAACGA-N₂-3' [Seq. ID No. 7] where N₁ and N₂ represent any sequence of nucleotides ranging in size from about seven to about ten nucleotides that target a specific RNA.

11. A cell having the plasmid of claim 8 transformed therein.

12. A single-stranded DNA enzyme comprising a 15 nucleotide catalytic domain flanked by random RNA target-binding domains of between about 7 and about 10 nucleotides each.

13. The single-stranded DNA enzyme of claim 12 wherein said catalytic domain comprises the sequence 5'-N₁-GGCTAGCTACAACGA-N₂-3' [Seq. ID No. 7], where N₁ and N₂ represent any sequence of nucleotides ranging in size from about seven to about ten nucleotides that target a specific RNA.

14. A plasmid having a sequence coding for the DNA enzyme of claim 12 contained therein.

15. A cell having the plasmid of claim 14 transformed therein.

16. The ODN expression plasmid AS080103.

17. A method of treatment of sepsis in an animal patient comprising the steps of contacting the causative agent of sepsis with an ODN comprising a sequence targeted to a specific gene of the causative agent for altering the expression of the specific gene to inhibit growth of the causative agent, kill the causative agent, or inhibit the synthesis or secretion of toxin by the causative agent.

18. A DNA, RNA, or PNA oligonucleotide for treatment of sepsis having one or more of the following nucleotide sequences:

5'(CTT TCA ACA GTT TTG ATG ACC TTT GCT GAC CAT ACA
ATT GCG ATA TCG TGG GGA GTG AGA G)3';

5'(CTC ATA CTC T)3'; or

5'(GTT TCG AAG GCT AGC TAC AAC GAT CAT CCA G)3'.

19. A cell having one or more of the sequences of claim 18 transformed therein.

20. An oligonucleotide having the sequence

5'-CTTTCAACAGTTTTGATGACCTTTGCTGACCATAACAATTGC-
GATATCGTGGGGAGTGAGAG-3' [Seq. ID No. 13].

Or one of the following sequences homologous thereto:

5'-CCTTTGCTGACCATAC-3'	[Seq. ID No. 14]
5'-GACCTTTGCTGACCA-3'	[Seq. ID No. 15]
5'-ACAGTTTTGATGAC-3'	[Seq. ID No. 16]
5'-ACAATTGCGATAT-3'	[Seq. ID No. 17]
5'-GACCTTTGCTGAC-3'	[Seq. ID No. 18]
5'-TCAACAGTTTTGATGAC-3'	[Seq. ID No. 19]
5'-ATGACCTTTGCTG-3'	[Seq. ID No. 20]
5'-CAGTTTTGATGA-3'	[Seq. ID No. 21]
5'-ACCTTTGCTGAC-3'	[Seq. ID No. 22]
5'-TTGCTGACCATA-3'	[Seq. ID No. 23]
5'-TGACCTTTGCTG-3'	[Seq. ID No. 24]
5'-GTTTTGATGACC-3'	[Seq. ID No. 25]
5'-GCGATATCGTGG-3'	[Seq. ID No. 26]
5'-TTGATGACCTTT-3'	[Seq. ID No. 27]
5'-TGGGGAGTGAG-3'	[Seq. ID No. 28]
5'-TTGCTGACCAT-3'	[Seq. ID No. 29]
5'-TTTTGATGACC-3'	[Seq. ID No. 30]
5'-TGATGACCTTT-3'	[Seq. ID No. 31].

21. A plasmid having a sequence coding for one or more of the oligonucleotides of claim 20 contained therein.

22. A cell having the plasmid of claim 21 transformed therein.

23. A single-stranded DNA enzyme comprising the nucleotide sequence 5'-N₁-GGCTAGCTACAACGA-N₂-3' [Seq. ID No. 7], and sequences homologous thereto, where N₁ and N₂ represent any sequence of nucleotides ranging in size from about seven to about ten nucleotides that are adapted for targeting a specific RNA.

24. The single-stranded DNA enzyme of claim 23 wherein N₁ and N₂ are comprised of a sequence specific for a sequence comprising a portion of the mRNA transcript of the bacterial FtsZ gene, and/or a sequence homologous thereto.

25. A plasmid having a sequence coding for the single-stranded DNA enzyme of claim 23 contained therein.

26. A peptide-PNA conjugate comprising the sequence KFFKFFKFFK CTC ATA CTC T [Seq. ID No. 34].

27. An oligonucleotide capable of binding to an mRNA transcribed from a cell growth, replication or toxin production or secretion gene of a bacterial or fungal pathogen, wherein the binding alters the activity of the gene.

28. An oligonucleotide according to Claim 27 wherein the oligonucleotide is selected from:

- | | |
|--|------------------|
| 5'-CTTCAACAGTTTGGATGACCTTTGCTGACCATAACAATTGC-GATATCGTGGGGAGTGAGAG-3' | [Seq. ID No. 13] |
| 5'-CCTTTGCTGACCATAC-3' | [Seq. ID No. 14] |
| 5'-GACCTTTGCTGACCA-3' | [Seq. ID No. 15] |
| 5'-ACAGTTTGGATGAC-3' | [Seq. ID No. 16] |
| 5'-ACAATTGCGATAT-3' | [Seq. ID No. 17] |
| 5'-GACCTTTGCTGAC-3' | [Seq. ID No. 18] |
| 5'-TCAACAGTTTGGATGAC-3' | [Seq. ID No. 19] |
| 5'-ATGACCTTTGCTG-3' | [Seq. ID No. 20] |
| 5'-CAGTTTGGATGA-3' | [Seq. ID No. 21] |
| 5'-ACCTTTGCTGAC-3' | [Seq. ID No. 22] |
| 5'-TTGCTGACCATA-3' | [Seq. ID No. 23] |
| 5'-TGACCTTTGCTG-3' | [Seq. ID No. 24] |
| 5'-GTTTGGATGACC-3' | [Seq. ID No. 25] |
| 5'-GCGATATCGTGG-3' | [Seq. ID No. 26] |
| 5'-TTGATGACCTTT-3' | [Seq. ID No. 27] |
| 5'-TGGGGAGTGAG-3' | [Seq. ID No. 28] |

5'-TTGCTGACCAT-3'

[Seq. ID No. 29]

5'-TTTTGATGACC-3'

[Seq. ID No. 30]

5'-TGATGACCTTT-3'

[Seq. ID No. 31]

29. An oligonucleotide according to Claim 27 or 28 wherein the binding prevents translation of the mRNA and the alteration is a reduction in the gene product.

30. An oligonucleotide according to any one of Claims 27-29 wherein the oligonucleotide is selected from single stranded DNA (ssDNA), RNA, peptide nucleic acids (PNA), locked nucleic acids (LNA), phosphorothioates or phosphorothioates morpholino oligo (PMO).

31. An oligonucleotide according to any one of Claims 27-30 wherein the pathogen is a sepsis causative agent.

32. An oligonucleotide according to any one of Claims 27-31 wherein the pathogen is selected from Gram-negative bacteria Bacteroides, Fusobacterium, Escherichia, Klebsiella, Salmonella, Shigella, Proteus, Pseudomonas, Vibrio, Legionella, Haemophilus, Bordetella, Brucella, Campylobacter, Neisseria, Branhamella, Gram-positive bacteria such as Streptococcus, Staphylococcus, Peptococcus, Bacillus, Listeria, Clostridium, Propionibacteria, organisms that stain poorly or not at all with Gram's stain Mycobacteria, Treponema, Leptospira, Borrelia, Mycoplasma, Chlamydia, Rickettsia and Coxiella, or fungi Candida, Aspergillus, Blastomycosis, Coccidioidomycosis, Cryptococcosis, Histoplasmosis, Paracoccidiomycosis, Sporotrichosis, Zygomycosis.

33. An oligonucleotide according to any one of Claims 27-32 wherein the gene is involved in one of cell division, cell wall synthesis, protein synthesis (translation), nucleic acid synthesis, fatty acid metabolism and gene regulation.

34. An oligonucleotide according to any one of Claims 27-33 wherein the mRNA has been transcribed from a gene selected from the bacterial genes FtsZ, MurB, acpP, 16s rRNA, PBPs, DNAA, DNAC, pcrA, rpoB, rpoA, rpoC, rpsC, rpsD, rpsF, rpsI, rpsJ, rpsM, rpsR, FabK, FabH, rplB, rplC, rplJ, rplK, rplM, rplN, rplO, rplP, rplR, rplT, rplV, rplX, rpmA, rpmL, valS, serS, proS, cysS, alaS, pheS, sporC, tsf, tufA, fus, secA, secV, pyrC, btuE, CaiB, ydgD, ygcQ, ftsH, ppiB, yihl, zntA, yicI, fhuA, rplD, ilvB, lepB, aroK, mfd, rlpA, accA, pgpA, or the fungal genes ERG1,

ERG2, ERG3, ERG4, ERG5, ERG6, ERG7, ERG11, ERG24, ERG25, ERGX, ERGY, CHS1, CHS2, CHS3, CWP1, CWP2, KRE1, KRE2, KRE5, KRE11, TIP1, GFA1.

35. An oligonucleotide according to any one of Claims 27-34 wherein the oligonucleotide functions as an antisense molecule.

36. An oligonucleotide according to any one of Claims 27-34 wherein the oligonucleotide functions as a DNA enzyme.

37. An oligonucleotide according to Claim 36 wherein the DNA enzyme is a 10-23 DNA enzyme.

38. An oligonucleotide according to Claim 37 wherein the DNA enzyme takes the form

5'-N₁-GGCTAGCTACAACGA-N₂-3' [Seq. ID No. 7]

where N₁ and N₂ represent any sequence of nucleotides that target a specific RNA ranging in size from 3 to 25 nucleotides, and preferably seven to ten nucleotides.

39. An oligonucleotide according to Claim 37 wherein the DNA enzyme is

5'-GTTTCGAAGGCTAGCTACAACGATCATCCAG-3' [Seq. ID No. 6]

40. An oligonucleotide according to Claim 39 wherein the DNA enzyme binds FtsZ mRNA.

41. A conjugate comprising an oligonucleotide according to any one of Claims 27 to 41 and a peptide.

42. A conjugate according to Claim 41 wherein the peptide is KFFKFFKFFK or homologues and derivatives thereof.

43. A vector comprising an oligonucleotide according to any one of Claims 27 to 41.

44. A vector according to Claim 43 wherein the vector is an expression vector, preferably an inducible expression vector.

45. A host cell transformed with a vector according to any one of Claims 43 to 44.

46. A host cell according to Claim 45 wherein the cell is a bacterial or fungal pathogen, preferably a sepsis causative agent.

47. An oligonucleotide according to any one of Claims 27 to 40, a conjugate according to Claim 41 or 42, or a vector according to Claim 43 or 44, for use in treatment.

48. An oligonucleotide according to Claim 47 wherein the treatment is treatment of a bacterial or fungal pathogenic condition and preferably sepsis.

49. Use of an oligonucleotide according to any one of Claims 27 to 40 in the manufacture of a medicament for the treatment of a bacterial or fungal pathogenic condition

50. Use according to Claim 49 wherein the condition is caused by Bacteroides, Fusobacterium, Escherichia, Klebsiella, Salmonella, Shigella, Proteus, Pseudomonas, Vibrio, Legionella, Haemophilus, Bordetella, Brucella, Campylobacter, Neisseria, Branhamella, Streptococcus, Staphylococcus, Peptococcus, Bacillus, Listeria, Clostridium, Propionibacteria, Mycobacteria, Treponema, Leptospira, Borrelia, Mycoplasma, Chlamydia, Rickettsia and Coxiella, or Candida, Aspergillosis, Blastomycosis, Coccidioidomycosis, Cryptococcosis, Histoplasmosis, Paracoccidiomycosis, Sporotrichosis, Zygomycosis.

51. Use according to Claim 49 or 50 wherein the condition is sepsis.

52. A kit comprising:

(a) an oligonucleotide according to any one of Claims 27 to 40;

and

(b) a pharmaceutically acceptable excipient

for use in treating a bacterial or fungal pathological condition.

53. A kit according to Claim 52 wherein the condition is sepsis.

54. A kit according to Claim 52 to 53 wherein the pharmaceutically acceptable excipient is selected from a peptide, preferably KFFKFFKFFK, and a vector.

55. A library comprising at least one bacterial strain transformed with an inducible expression vector comprising a test oligonucleotide, wherein the bacterial strain is capable of causing a pathological condition.

56. A library according to Claim 55 wherein the pathological condition is sepsis.

57. A library according to Claim 55 or 56 wherein the bacterial strain is selected from Gram-negative bacteria *Bacteroides*, *Fusobacterium*, *Escherichia*, *Klebsiella*, *Salmonella*, *Shigella*, *Proteus*, *Pseudomonas*, *Vibrio*, *Legionella*, *Haemophilus*, *Bordetella*, *Brucella*, *Campylobacter*, *Neisseria*, *Branhamella*; Gram-positive bacteria *Streptococcus*, *Staphylococcus*, *Peptococcus*, *Bacillus*, *Listeria*, *Clostridium* and *Propionibacteria*.

58. A library according to any one of Claims 55 to 57 wherein the bacterial strain is *E. coli*, preferably DH5 α Pro cells.

59. A library according to any one of Claims 55 to 58 wherein the test oligonucleotide is at least 10, preferably 20, more preferably 30, still more preferably 40, even more preferably 50 and still even more preferably 60 bases long.

60. A library according to any one of Claims 55 to 59 wherein the inducible expression vector is modulated by tetracycline and preferably the vector is pssXGb.

61. A method for identifying an oligonucleotide for use in the treatment of a bacterial or fungal pathological condition comprising:

- (a) providing a library according to any one of Claims 55 to 60;
- (b) providing at least two samples of the at least one bacterial strain, under conditions supporting bacterial growth, wherein at least one sample is contacted with the inducer and at least one sample is not contacted with the inducer; and
- (c) selecting a bacterial strain capable of growing only in the absence of the inducer and isolating the test oligonucleotide.

62. A method according to Claim 61 wherein the condition is sepsis.

63. A method according to Claim 61 or 62 further comprising step (d) to be performed after step (c) comprising:

- (d) analysing the isolated test oligonucleotide to identify a target bacterial gene sequence.

64. A method according to any one of Claims 61 to 63 further comprising step (e) to be performed after step (c) or (d) comprising:

- (e) use of the isolated test oligonucleotide in a treatment for sepsis.

65. A method according to any one of Claims 61 to 64 wherein the bacterial strain is selected from *Bacteroides*, *Fusobacterium*, *Escherichia*, *Klebsiella*,

Salmonella, Shigella, Proteus, Pseudomonas, Vibrio, Legionella, Haemophilus, Bordetella, Brucella, Campylobacter, Neisseria, Branhamella; Streptococcus, Staphylococcus, Peptococcus, Bacillus, Listeria, Clostridium, Propionebacteria.